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EVOLUTIONARY RELATIONSHIP OF NUCLEAR GENES ENCODING MITOCHONDRIAL PROTEINS ACROSS FOUR GRASS SPECIES AND *ARABIDOPSIS THALIANA*

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ABSTRACT - Comparative genomics studies across taxa provide basic understanding of genome evolution. This study focused on nuclear genes encoding mitochondrial proteins across *Arabidopsis* and several grass species. Two different mitochondria-related gene sets, one rice-derived and another *Arabidopsis*-derived, were used in our analysis. Eleven of the rice-derived genes, from a 6.85 Mb region of chromosome 10, were physically mapped using radiation hybrid (RH) approach on chromosome 1D of wheat. Based on the comparative analysis of these 11 genes, genomes of wheat and maize shared more comparable pattern of conservation than wheat and rice. Additionally, rice, revealed to have perfectly conserved colinearity with *Brachypodium*, whereas, wheat and *Brachypodium* showed less preserved synteny. This was surprising since previous studies have estimated that *Brachypodium* was evolutionarily closer to wheat than rice when comparing their divergence times. Some rearrangements in studied regions of wheat and maize genomes may indicate a relaxation of evolutionary pressure on gene order in polyploid as compared with diploid species. Additionally, our cluster analysis on chromosome 10 of rice identified two mitochondria-related gene clusters, one containing seven and another one two genes. Analysis of *Arabidopsis*-derived set of 473 genes showed 14 to 16% conservation in rice, maize, *Brachypodium*, and wheat. Forty five genes showed to be conserved in representatives of monocots as well as in *Arabidopsis* which underlies the significance of their conservation for survival of plant mitochondria. Moreover, observed clustering of the mitochondria-related nuclear genes on chromosome 10 of rice indicates that the gene clustering might be one of the ways to preserve crucial genes from major rearrangements or recombination events that could compromise their function.

KEY WORDS: Mitochondria-related genes; Colinearity; Radiation hybrid mapping.

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INTRODUCTION

Comparative genomics, involving analysis of genome organization in related species, can be used for better insight as to the evolution and organization of genes and for transfer of sequence information from better studied model species to related organisms. Macro-colinearity of grass genomes, defined as a conservation of the gene/marker content and order at the chromosomal level between various Poaceae species, has been extensively studied for the last 20 years. Despite a 35-fold variation in genome size of grasses, colinear relationships were discovered. Different studies comparing the important grass species, such as wheat, maize, rice, sorghum, sugarcane, and millet have resulted in models of genomes that could be aligned to form the so called "grass circle" (GALE and DEVOS, 1998).

Recent comparative analyses based on rice genome sequence information and physically mapped wheat Expressed Sequence Tag (EST) markers have shown a pattern that most rice chromosomes are related to a single chromosome of wheat (with short and long arms matching different arms of the same wheat chromosome), whereas wheat chromosomes are composed of linkage blocks corresponding to one, two or three rice chromosomes (SORRELLS *et al.*, 2003; LA ROTA and SORRELLS, 2004). Moreover, a loss of colinearity within centromeric and telomeric regions was observed. It was speculated that this tendency was due to a sequence gap in rice centromeric sections, or lack of wheat ESTs representing the centromeric sequences. The other hypothesis was that these regions were prone to rearrangements as they were rich in repetitive sequences and transposons (LA ROTA and SORRELLS, 2004).

A collection of more complete and accurate sequence data led to more detailed studies on levels

of micro-colinearity, defined as a conservation of the gene/marker content and order at the sequence level. A general trend for better conservation in proximal regions of wheat chromosomes in comparison to distal ones was observed. An example of well conserved micro-colinearity is the 556-Kb region of wheat chromosome 5AL spanning the vernalization (*VRN1*) gene and its homologous regions in rice and sorghum (YAN *et al.*, 2003). On the other hand, regions of severely disrupted micro-colinearity have also been discovered. An example of low similarity is wheat chromosome 5BL region carrying *Tsn1* gene, coding sensitivity to a toxin produced by the tan spot fungus, and rice chromosome 9 (LU and FARIS, 2006).

More recently *Brachypodium* has become an important species in comparative analyses within the Triticeae tribe. *Brachypodium distachyon*, commonly called purple false brome, has all the characteristics of a model species including a small genome size (~355 Mb), self-fertility, a short life cycle (11-18 weeks), simple growth conditions and an easy genetic transformation system (DRAPER *et al.*, 2001; CHRISTIANSEN *et al.*, 2005; VOGEL *et al.*, 2006). Phylogenetic studies have shown that *Brachypodium* is more closely related to wheat in comparison to rice making it an ideal model grass for comparative genomics (CATALAN and OLMSTEAD, 2000; GPWG, 2001; BOUCHENAK-KHALLADI *et al.*, 2008).

HUO *et al.* (2007) created and analyzed two BAC libraries of *Brachypodium*, from which 2,185 BAC end sequences (BES) spanning 1.3 Mb of random sequences were generated and characterized. Only 3.3% of these sequences showed matches with repetitive elements providing an evidence of lower genome repetitiveness in comparison to other grasses. This could also explain the small genome size of *Brachypodium* as compared with other grasses. Additionally, these BES were used to screen available EST databases of wheat and maize resulting in more hits with wheat ESTs than maize, again pointing out the close relationship of these two species. Another study has been performed on *B. sylvaticum* and *B. distachyon* and focused on a 371-Kb region compared with the orthologous regions from rice and wheat. These results demonstrated that *Brachypodium* in this region had almost 50% and 40% fewer genes than rice and wheat, respectively. In this region there was a perfect macro-colinearity between *Brachypodium* and wheat, while there was a 220-Kb inversion relative to rice. The divergence time of *Brachypodium* and wheat has been estimated to be around 35-40 Million Years Ago (MYA), which was more recent than the divergence of rice and wheat which occurred around 50 MYA (PATERSON *et al.*, 2004; BOSSOLINI *et al.*, 2007).

Recent comparison of *B. distachyon* BES with 12 rice pseudo-molecules representing ~1 Mb sequence from nine BAC clones of rice revealed both well conserved regions as well as regions with disrupted micro-colinearity due to insertions/deletions, inversions, and differential duplications (HUO *et al.*, 2009). It was discovered that 17% of genes have no colinearity within orthologous areas. Despite a certain amount of disrupted micro-colinearity between *Brachypodium*, wheat and rice, *Brachypodium* has shown to have a potential to become a new model species for grass family. Thus, the complete genome sequence of this species provides valuable information for evolutionary studies between grasses, new markers for positional cloning, as well as help in assignment of wheat ESTs to particular deletion bins (HUO *et al.*, 2009).

In this study we focused initially on a 6.85 Mb-region of chromosome 10 of rice. This region has been shown to be colinear with chromosome 1D of wheat where a gene for nuclear-cytoplasmic interaction is located (MAAN *et al.*, 1999). We are interested in nuclear-cytoplasmic interaction studies which have been shown to be critical to plant development and survival (MAAN, 1991, 1992; KALAVACHARLA *et al.*, 2006). The goal of our initial study was to analyze nuclear-encoded mitochondrial genes within this region and the colinearity of these genes among grasses. The conservation within this region led to analysis of pattern of these genes across the entire genome of several grass species. We took 473 nuclear genes from *Arabidopsis thaliana,* which were predicted to be mitochondria-related, in order to obtain the most complete and representative set for our analysis (ALEXEYENKO *et al.*, 2006). The objectives of this study were to determine if a conserved colinearity of nuclear-encoded mitochondrial genes exists across different grass species; as well as to determine if there are any nuclear-encoded mitochondrial gene clusters.

MATERIALS AND METHODS

Radiation hybrid mapping population development

Seeds for 'Langdon' (LDN) substitution line 1D (1A), where a pair of chromosome 1D from *Triticum aestivum* (2n=6x=42, AABBDD) has been substituted for chromosome 1A of durum wheat (*T. turgidum*; 2n=4x=28; AABB), generated by JOPPA and

WILLIAMS (1988) were obtained from Dr. S. Xu at USDA-ARS Fargo North Dakota. About 100 seeds of LDN 1D (1A) were irradiated with 150 Gy γ rays. Subsequently, irradiated seeds were grown and surviving plants were backcrossed to a normal LDN durum line giving rise to the RH_1 population consisting of 94 independent radiation hybrid lines (RH panel). DNA from these lines was extracted using the isolation protocol, previously described by HOSSAIN *et al.* (2004), and used as a mapping population for screening with molecular markers.

Chromosome 10 of rice gene-based markers

A 'MapViewer' option at NCBI (http://www.ncbi.nlm.nih.gov/) was used to identify mitochondrial genes on chromosome 10 of rice which was found to be mostly collinear with chromosome 1D of wheat. This search resulted with 11 nuclear-encoded mitochondrial targeted protein genes and with 24 PPR/TPR-like genes, which are believed to be connected to organelle-related functions (LURIN *et al.*, 2004). These 24 genes were screened with 'Mitopred', a free online software program to predict which nuclear genes encode for mitochondrial proteins (http://bioapps.rit.albany.edu/MITOPRED). Seventeen genes which were confirmed by this method to be mitochondria-related were used to design wheat primers. The rice gene sequences were used to find wheat homologous tentative consensus sequences (TCs) using BlastN available on TIGR.org (http://compbio.dfci.harvard.edu/cgi-bin/tgi/gimain.pl?gudb=wheat). Tentative consensus sequences with a cut off e-value of 1.0 e-30 or lower were incorporated into our analysis. Finally, genomic and mRNA sequences of rice genes and homologous wheat TCs were aligned using ClustalW2 software (http://www.ebi.ac.uk/Tools/clustalw2/index.html) to identify sequences of exons and introns. If possible exon sequences bordering introns were used to design primers. Primers were designed for the 28 mitochondria-related nuclear genes, at least three pairs of primers per gene, using Primer3 software (http://frodo.wi.mit.edu/). Subsequently, the mentioned above primer pairs were used to screen RH panel for chromosome 1D of wheat to map and order these genes on this chromosome.

Arabidopsis thaliana *nuclear-encoded mitochondrial gene set*

A set of 473 nuclear genes was adopted for our study from ALEXEYENKO *et al.* (2006). *Arabidopsis thaliana* coding regions (CDS) were compared with maize, rice, wheat, and *Brachypodium.* The 473 nuclear gene sequences were compared against available sequenced genomes and reference sources using the BlastN algorithm. We applied a cut off value of BlastN similarity score of 100 and higher to find orthologs across the grasses as it had been used before to find the duplicated genes in *Arabidopsis* (ALEXEYENKO *et al.,* 2006). Plant genome sequences were available for the *Arabidopsis* (NCBI Genbank), rice (TIGR version 5), and *Brachypodium* (JGI, pre-release) genomes. Comparisons were also made against maize BACs (www.maize.org) which were ordered onto genome coordinates based on the assembly data available. Also used as DNA resources were mapped sequences of wheat (LAZO *et al.*, 2004). Other searches were against Genbank non-redundant (http://www.psc.edu/general/software/packages/genbank/genbank.php), dbEST (http://www.ncbi.nlm.nih.gov/dbEST/), and GIRI repetitive sequence databases (http://www.girinst.org/about/index.html).

Screening with markers

PCR reactions were performed in a total volume of 20µl in a model 9700 and 2720 thermocyclers (Applied Biosystems, Foster City, CA). The reaction mixture contained 250 nM of each primer, 0.2 mM of each deoxynucleotide, 1.5 mM $MgCl_2$, 1 unit *Taq* polymerase, and 50 ng of template DNA. A touchdown PCR reaction was conducted with initial denaturing of 94°C for 4 minutes, following by 10 cycles of 94°C for 30 sec., 65°C for 30 sec. (reduced by 0.5°C each cycle) and 1 min. of extension at 72°C, and 35 cycles of the same conditions except the annealing temperature was fixed at 60°C, and a final extension of 72°C for 10 min. Amplified products were separated using non-denaturing 6% polyacrylamide gel electrophoresis using ethidium bromide staining (KALAVACHARLA *et al*., 2006).

Generation of wheat 1D RH map

A radiation hybrid map of chromosome 1D was created based on mapping data generated from 53 chromosome-specific molecular markers using Carthagene 0.999 (http://www.inra.fr/bia/T/Carthagene). As previously mentioned markers designed from nuclear genes encoding mitochondria-related proteins were used in the screening. Additionally, markers of various types (SNP derived from ESTs, ESTs, and retrotransposon junction markers) were used in order to create a complete physical map (data not shown). Carthagene 0.999 (http://www.inra.fr/mia/T/CarthaGene/) mapping software was used to generate RH map with linkage group verified with a command at two point distance of 80 cR (centiRays) and a LOD score of 4.00 as threshold values. A build command, a heuristic approach where markers are progressively added, was used to build a map. Finally, annealing, flips and polish commands were applied to improve the map, validate the marker order of the best map. A map with the best likelihood was identified using heap command.

Chromosome 10 of rice gene cluster analysis

A gene cluster was defined as a group of non-randomly distributed genes on a chromosome. To validate whether mitochondria-related genes on chromosome 10 were clustered or not, Chi-square analysis of ratios derived from gene densities was performed. This analysis compared density of all genes vs. mitochondria-related density of genes to evaluate randomness of distribution.

RESULTS

The region on chromosome 10 of rice

For the purpose of comparative analysis between *A. thaliana*, *Brachypodium*, rice, maize, and wheat, 11 out of 28 nuclear-encoded mitochondrial genes identified on rice chromosome 10 were physically mapped to wheat chromosome 1D. These genes represent 6.85 Mb-region in rice, 8.06 Mb in *Brachypodium* (~1.176 times larger), 19.1 Mb region on chromosome 5 of *A. thaliana* (~2.8x larger), 205.6 Mb on chromosome 1 of maize (~30x larger), and approximately ~9.1 Mb on chromosome 1D of wheat (~1.3x larger). The rice genes have orthologs on chromosomes 1, 2, 4 and 5 of *A. thaliana* (Fig. 1). However, majority of these genes (6/11 = 54.5%) have orthologs located on chromo-

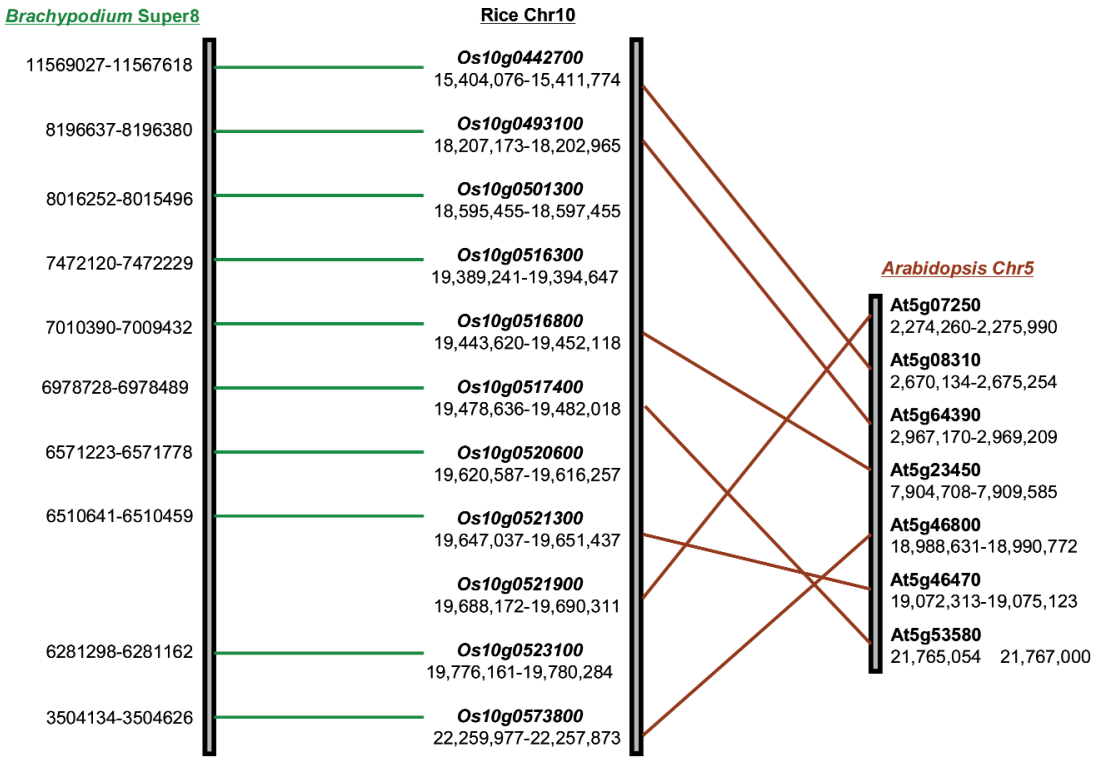

FIGURE 1 - Colinearity of 11 mitochondria-related nuclear genes between *Brachypodium*, rice chromosome 10, and *Arabidopsis* chromosome 5. Localization of genes on a particular chromosome is expressed in base pairs (bp).

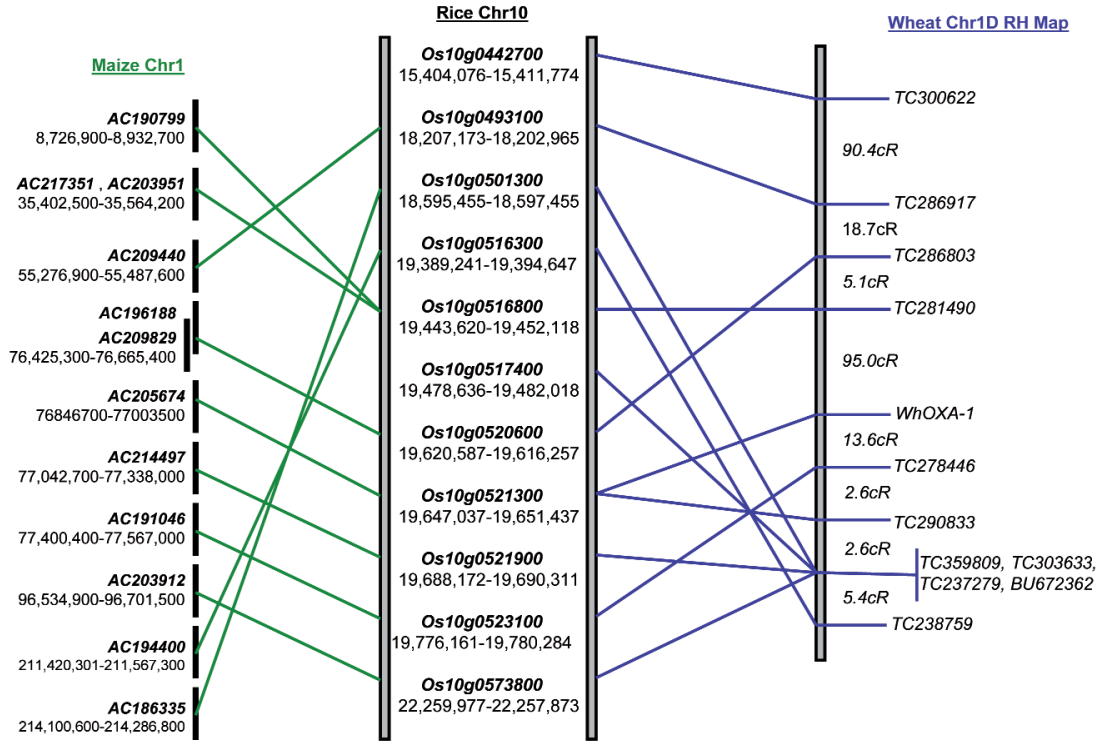

FIGURE 2 - Colinearity of 11 mitochondria-related nuclear genes between maize chromosome 1, rice chromosome 10, and physical map of wheat chromosome 1D. Localization of genes on a particular chromosome is expressed in base pairs (bp).

TABLE 1 - *Eleven mitochondria-related nuclear genes of rice and their orthologs on maize chromosomes.*

	Rice gene ID	Maize hit similarity (protein)*	Maize BAC	Maize chromosome (hit e value)	Location on a chromosome
1	*Os10g0442700*	39%	AC204746	Chr9 (0.0)	120035300-120152900
			AC191275	Chr5 (9E-20)	119168000-119300300
			AC203167	Chr5 (9E-20)	119266000-119462000
			AC190839	Chr1 (1E-09)	5968200-6085800
			AC194223	Chr7 (2E-05)	651700-798700
			AC225565	Chr7 (2E-05)	460600-671300
2	*Os10g0493100*	30%	AC209440	Chr1 (0.0)	55276900-55487600
			AC217659	Chr2 (0.0)	106516200-106673000
			AC206245	Chr6 (3E-13)	148127000-148239700
			AC230036	Chr6 (3E-13)	148043700-148166200
		30%	AC190901	Chr2 (0.0)	208328400-208455800
			AC177865	Chr2 (0.0)	208504800-208686100
3	*Os10g0516300*	89%	AC194400	Chr1 (1E-67)	211420301-211567300
4	*Os10g0501300*	58%	AC191072	Chr2 (0.0)	61896800-62073200
			AC186335	Chr1 (0.0)	214100600-214286800
			AC196432	Chr5 (7E-11)	22966300-23172100
			AC204348	Chr5 (7E-11)	23191700-23343600
5	*Os10g0516800*	64%	AC200508	Chr10 (0.0)	117712700-117854800
			AC205708	Chr4 (0.0)	192643500-192805200
			AC208120	Chr4 (0.0)	96397700-96554500
			AC217351	Chr1 (5E-72)	35402500-35564200
			AC203951	Chr1 (5E-72)	35402500-35564200
			AC190553	Chr9 (5E-63)	94873800-95089400
		64%	AC190799	Chr1 (5E-72)	8726900-8932700
			AC205266	Chr3 (E-168)	201796700-201953500
			AC204651	Chr5 (E-115)	167379100-167521200
			AC210196	Chr5 (E-115)	167237000-167418300
			AC204651	Chr5 (E-115)	167379100-167521200
6	*Os10g0517400*	40%	AC201787	Chr2 (2E-99)	29302000-29444100
			AC186333	Chr2 (2E-99)	29351000-29522500
7	*Os10g0520600*	91%	AC191557	Chr9 (0.0)	122426500-122627400
			AC196000	Chr9 (0.0)	122308900-122480400
			AC196188	Chr1 (0.0)	76425300-76621300
			AC209829	Chr1 (0.0)	76464500-76665400
8	*Os10g0521300*	70%	AC205674	Chr1 (0.0)	76846700-77003500
9	*Os10g0521900*	90%	AC214497	Chr1 (0.0)	77042700-77338000
10	*Os10g0523100*	77%	AC196000	Chr9 (9E-99)	122308900-122480400
			AC191046	Chr1 (3E-42)	77400400-77567000
11	*Os10g0573800*	89%	AC203912	Chr1 (0.0)	96534900-96701500

* Rice genes where translated into protein sequences and compared with maize database using NCBI BLAST tool (http://www.ncbi.nlm.nih.gov/).

some 5 of *A. thaliana*. Comparison between rice and the *Brachypodium* Super8 contig indicates an almost perfect colinearity (10/11 = 90.9% of genes show a linear relationship) (Fig. 1). One of the genes could not be found on Super8 contig indicating either its deletion or translocation into a new non-orthologous region in the *Brachypodium* genome.

The analyzed 6.85 Mb-region of rice is colinear with three chromosomes of maize (chromosome 1,

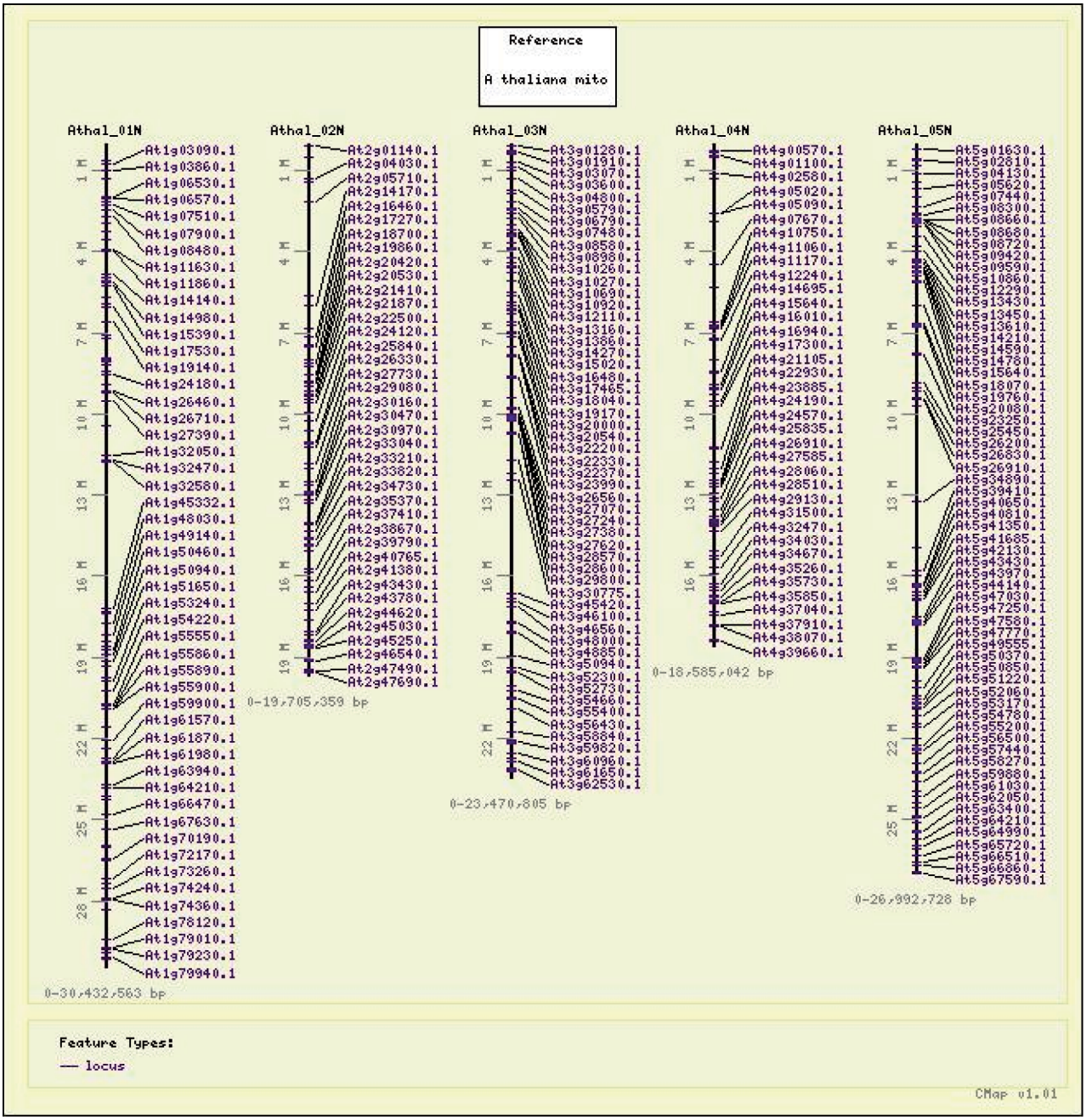

FIGURE 3 - Graphic representation of 473 mitochondria-related genes used in this study and their locations on five chromosomes of *Arabidopsis*.

2, and 9). Ten rice genes had their orthologs on chromosome 1, four on chromosomes 9 and 2, three on chromosome 5, and one on chromosomes 3, 4, and 7 of maize. Although, there are more rice orthologs located on other maize chromosomes (Table 1), only chromosome 1, with the highest number of rice orthologs, was presented on Fig. 2. All of the rice genes have orthologs on chromosome 1D of wheat (Fig. 2). There were six genes (6/11=54.5%) with conserved order between these two species. Four of the 11 genes mapped to the same region. These loci could not be ordered using the RH panel since individuals of this panel did not have enough breaks to resolve the order of these genes.

TABLE 2 - *Number of the* Arabidopsis *mitochondria-related nuclear genes conserved across the genome of maize, rice,* Brachypodium, *and wheat.*

	Arabidopsis	**Maize**	**Rice**	***Brachypodium***	**Wheat**
Chr. 1	92	7	8	5	3
Chr. 2	77	14	14	9	7
Chr. 3	107	12	14	15	12
Chr. 4	72	13	15	11	8
Chr. 5	125	23	26	25	18
Total	473	70	77	65	49

The numbers in the table specify the number of the mitochondria-related nuclear genes from five chromosomes of *Arabidopsis* as well as the number of their orthologs in the four grass species. The 473 genes from *Arabidopsis* are all adopted from ALEXEYENKO *et al.* (2006). The orthologous genes were found based on the Blast similarity score of 100 or higher using BlastN search.

In wheat, the analyzed 233.4 Centi-Ray (cR)-region, is colinear with mostly three chromosomes of maize, chromosome 1, 2, and 9 (Fig. 2). Nine wheat TCs have their orthologs on chromosome 1, four on chromosomes 9 and 2, three on chromosome 5, and one on chromosomes 3, 4, and 7 of maize. Again six genes of wheat have a single ortholog in maize, and five of the genes have at least two orthologs on different maize chromosomes.

Rice chromosome 10 genes set cluster analysis

To determine whether mitochondria-related genes on chromosome 10 were clustered, the total number of genes and mitochondria-related genes for a given region were determined and divided by the total number of those two gene classes on the entire chromosome, respectively, providing a measure of gene density. These densities were then compared using the Chi-square analysis to determine significant deviations from the expected 1:1 ratio. Total number of genes for the entire chromosome 10 is 1,591, for the studied 6.85 Mb is 746, for the 4.06 Mb is 492, for 0.394 Mb is 53, and 0.391 Mb is 41 genes. Total number of mitochondria-related genes for the entire chromosome 10 is 23, for 6.85 Mb is 11, for 4.06 Mb is 10 genes, for 0.394 is 2, and 0.391 Mb is 7. Chi-square test indicated that mitochondria-related genes on the entire chromosome 10 are randomly distributed. However, two gene clusters were identified representing a cluster of 7 genes (Os10g0516300, Os10g0516800, Os10g0517400, Os10g0520600, Os10g0521300, Os10g0521900, and Os10g0523100) within 19,389,241 bp - 19,780,284 bp and 2 genes (Os10g0493100 and Os10g0501300) within the 18,202,965 bp - 18,597,455 bp region of rice chromosome 10.

Mitochondria-related gene set from Arabidopsis

To have a better idea of the conservation of mitochondria-related nuclear genes across the grass genome the list of 473 genes, Fig. 3, published by ALEXEYENKO *et al.* (2006) was taken for detailed analysis. BlastN was used to find orthologous sequences of the 473 genes in four grass species (Table 2). About 14 to 16% of the *Arabidopsis* mitochondria-related genes had orthologs in *Brachypodium*, rice and maize (Table 2; Fig. 4). Only 5 to 9% of the genes from chromosome 1 of *Arabidopsis* had matches with genes in grasses but the genes from chromosomes 4 and 5 of *Arabidopsis* had matches ranging from 15 to 20% and 18 to 20%, respectively. Chromosome 5 of *Arabidopsis* carries 26% of the 473 mitochondria-related genes while chromosome 4 carries the least (15%). Among all the genes analyzed (excluding data from wheat due possibly to low EST representation), 45 genes were conserved in representatives of monocots as well as in *Arabidopsis* (Table 3).

DISCUSSION

Mitochondria are believed to originate from α-proteobacteria which through endosymbiotic events were incorporated into eukaryotic cell. Throughout evolution of eukaryotes most of the essential genes involved in function of mitochondria have been transferred to the nucleus leading to shrinkage of the mitochondrial genome and making the nuclear genome even more complex (TIMMIS *et al.*, 2004). Due to this exchange of information a proper crosstalk between these two organelles is essential for a survival of any organism.

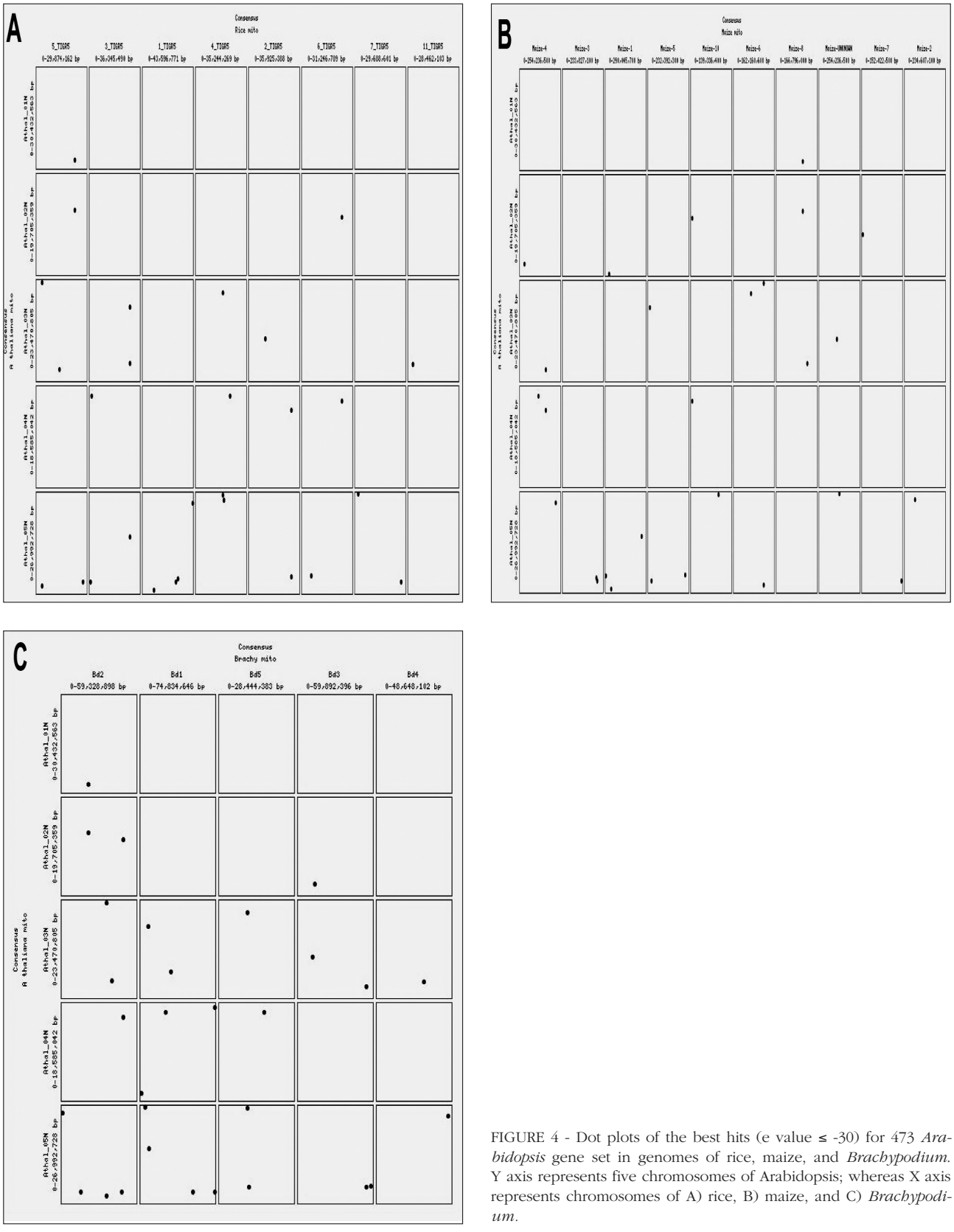

FIGURE 4 - Dot plots of the best hits (e value ≤ -30) for 473 *Arabidopsis* gene set in genomes of rice, maize, and *Brachypodium*. Y axis represents five chromosomes of Arabidopsis; whereas X axis represents chromosomes of A) rice, B) maize, and C) *Brachypodium*.

Recent studies have shown that mitochondria-related nuclear genes have a tendency to be clustered (ALEXEYENKO *et al.*, 2006). These clusters are small and scattered, without unusually increased co-expression or conserved syntenic patterns, indicating that such clustering could be a result of "continuous selection favoring chromosomal proximity of genes acting in the same organelle" (ALEXEYENKO *et al.*, 2006). There are other theories why the mitochondria–related nuclear genes could cluster together. One hypothesis is that they represent an original insertion site of mitochondrial genes into the nuclear genome. This could be confirmed if there was an increased colinearity between several species. If such similarity was not observed, gene clusters could not be considered remnants of such insertion events (ALEXEYENKO *et al.*, 2006). Another hypothesis is that the non-random gene distribution could be related to an aspect of gene regulation for genes that share a functional relationship. Such gene clusters simply would be easier to control if they were in the neighboring regions analogous to cases of prokaryotic operons (FIRNEISZ *et al.*, 2003; LEE and SONNHAMMER, 2003). Finally, this could also be connected to survival of gene groups from recombination as well as whole genome rearrangements like in the case of decreased recombination rate within clusters of important genes in yeast (PAL and HURST, 2003).

This study focused on two data sets of mitochondria-related nuclear genes, first set of genes identified on chromosome 10 of rice, and a second set of genes identified in the *Arabidopsis thaliana* genome. The objectives were to determine if a conserved colinearity of nuclear-encoded mitochondrial genes exists across different grasses, as well as if any clustering of nuclear-encoded mitochondrial genes is present in the studied region of rice.

Physical mapping results indicate that rice chromosome 10 region and the *Brachypodium* Super8 contig are perfectly collinear. However, when comparing this region of rice with *A. thaliana,* maize, and wheat, chromosomal rearrangements, such as inversions and translocations were observed, disrupting the order of the mitochondria-related genes. Since *Arabidopsis* underwent three whole-genome duplication events (the most recent 80-20.4 MYA, older one which post-dated divergence from the monocots about 170-235 MYA, and the oldest duplication which pre-dated monocot-dicot divergence 300 MYA) followed by genome diploidization and shuffling, it is not surprising that the colinearity with rice was disrupted (BOWERS *et al.*, 2003). But considering the perfect colinearity between *Brachypodium* and rice, lack of such a relationship with wheat and maize presents a glimpse into comparative evolutionary changes in genomes of these important grasses.

The colinearity map of grass genomes, the "grass circle", indicates that rice chromosome 10 is collinear with group 1 chromosomes of wheat, as well as chromosomes 1 and 5 of maize (GALE and DEVOS, 1998). Interestingly, we found six out of 11 genes of rice to have a single ortholog in maize, and five to have at least two orthologs on different maize chromosomes. This suggests duplication of certain segments within the maize genome which is in agreement with previous findings indicating that maize is an ancient polyploid which has undergone a relatively recent genome-wide duplication ~11 MYA (PATERSON *et al.*, 2004). Additionally, the syntenic region in maize was much bigger than rice and *Brachypodium,* suggesting high repetitive sequence content in maize within the studied area.

Comparison of wheat with rice or *Brachypodium* also indicates changes in colinearity arrangement of these 11 loci. This is not surprising as polyploidy in wheat allows for changes in order, deletions and duplications of loci without any functional detriment. However, the preservation of all these loci on chromosome 1D is significant in wheat as functional importance of these loci is still not lost. The interesting conclusion from this study is that both maize and wheat have retained the majority of these mitochondria-related genes from the ancestral monocot but have allowed certain changes in their organization due to either ancient or more recent polyploidy events. Wheat chromosome 1D, which belongs to the most recently added to wheat D genome, has retained these loci on a syntenic segment with few minor changes. It would be interesting to analyze the distribution of these loci in *T. turgidum* (AABB) where the two genomes have coexisted longer, A and B genomes hybridized <0.5 MYA, than the recent addition (~8,000 years ago) of the D-genome in *T. aestivum* (FELDMAN *et al.*, 1995; SALSE *et al.*, 2008).

Wheat underwent relatively recent polyploidy events that occurred within 5 MYA (ADAMS and WENDEL, 2005), whereas maize underwent more ancient polyploidization 11.4 MYA (GAUT and DOEBLEY, 1997). Polyploidization of whole genomes usually is followed by differential gene loss (diploidization) from redundant genomic regions as well as many

TABLE 3 - *List of the* Arabidopsis *mitochondria-related nuclear genes conserved in genomes of maize, rice,* Brachypodium, *and wheat.*

	Arabidopsis	**Maize**	**Rice**	***Brachypodium***	**Wheat**
1	At1g07510.1	AC193772.2-Contig21	AC105770 (Chr_05N)	Bd2(20944304-20943785)	
2	At1g09210.1	AC202976.2-Contig46	AP003812 (Chr_07N)		
3	At1g16700.1		AC092781 (Chr_03N)	Bd1(4996198-4996290)	
4	At1g17290.1	AC211172.1-Contig51			
5	At1g24180.1				BF483643
6	At1g29880.1	AC210078.1-Contig19	AP004464 (Chr_08N)		
7	At1g45332.1	AC206531.1-Contig32	AC084764 (Chr_03N)		
8	At1g50200.1	AC190630.2-Contig45			
9	At1g53240.1		AC104284 (Chr_05N)	Bd2(13933796-13933938)	BE490592
10	At1g59900.1				BF483643
11	At1g69220.1		AC120509 (Chr_03N)	Bd1(5825190-5825096)	
12	At1g70320.1		AP005972 (Chr_09N)		
13	At1g79010.1	AC194072.2-Contig16		Bd1(4996201-4996286)	
14	At2g01140.1	AC177914.2-Contig271	AP002541 (Chr_01N)	Bd2(841610-841798)	
15	At2g05710.1	AC200293.3-Contig33	AC118132 (Chr_03N)	Bd3(13334098-13334378)	BE490704
16	At2g13560.1	AC186831.3-Contig22	AP003956 (Chr_07N)	Bd1(22083959-22083887)	
17	At2g17130.1	AC205538.1-Contig28	CR855156 (Chr_04N)		
18	At2g18450.1	AC205479.1-Contig55	OSJN01002 (Chr_07N)	Bd1(4910908-4911062)	BQ281066
19	At2g20420.1	AC187833.2-Contig39	AP004053 (Chr_02N)	Bd3(50465110-50465257)	BE404354
20	At2g21410.1	AC206626.1-Contig40		Bd1(66515748-66515604)	
21	At2g24120.1	AC201907.3-Contig19	AP005729 (Chr_09N)		
22	At2g26080.1	AC198371.2-Contig31	AP003616 (Chr_06N)	Bd2(48357312-48357500)	
23	At2g29080.1	AC193772.2-Contig21	AC105770 (Chr_05N)	Bd2(20944328-20943655)	
24	At2g30970.1		AP004731 (Chr_06N)		BE426857
25	At2g33040.1		AE017074 (Chr_10N)		
26	At2g33210.1	AC186905.2-Contig35	AE017102 (Chr_10N)	Bd3(29415746-29415849)	BE518255
27	At2g38670.1	AC206919.1-Contig14	CNS08CDW (Chr_12N)		
28	At2g44350.1				BF291471
29	At2g45030.1	AC206531.1-Contig32			
30	At2g47510.1				BE403167
31	At3g02090.1				BE442995
32	At3g08580.1	AC186512.3-Contig12	AC098572 (Chr_05N)	Bd3(54189994-54190203)	BE426214
33	At3g09810.1	AC186308.3-Contig36			
34	At3g11670.1	AC213537.1-Contig32	AC133217 (Chr_11N)	Bd4(30242839-30243056)	BF428978
35	At3g12110.1	AC198971.2-Contig31	AC091532 (Chr_03N)	Bd2(39747518-39746916)	BE490281
36	At3g14270.1			Bd1(59719997-59720107)	
37	At3g15020.1		AP003053 (Chr_01N)	Bd2(45584271-45584409)	BE490592
38	At3g17240.1			Bd2(10639087-10638837)	
39	At3g18040.1	AC212045.1-Contig28	AP003621 (Chr_06N)	Bd1(30309164-30309435)	BE495644
40	At3g19170.1			Bd3(57704298-57704202)	BE490226
41	At3g20540.1		AP003882 (Chr_08N)		
42	At3g23990.1	AC196086.2-Contig34	AE017102 (Chr_10N)	Bd3(29419146-29419240)	BE518255
43	At3g26560.1	AC206665.1-Contig62	AP005532 (Chr_02N)	Bd3(11909353-11910190)	BG314071
44	At3g27240.1	AC211139.1-Contig106	AC093953 (Chr_05N)	Bd3(44182307-44182579)	BE404540
45	At3g27560.1				BE425967
46	At3g46520.1	AC193442.2-Contig34	AC091532 (Chr_03N)	Bd1(7692887-7692273)	BE490281
47	At3g48680.1		AP005066 (Chr_02N)		
48	At3g52300.1			Bd1(24107205-24107304)	
49	At3g55410.1	AC208928.1-Contig40	OSJN00244 (Chr_04N)	Bd5(10802756-10803069)	
50	At3g59820.1	AC212562.1-Contig61	OSJN00052 (Chr_04N)		
51	At3g61650.1	AC205034.2-Contig47	AC087425 (Chr_05N)	Bd2(35019318-35018913)	
52	At4g02930.1		AC096688 (Chr_03N)	Bd1(1032297-1032499)	
53	At4g08390.1	AC194336.2-Contig18	CR855188 (Chr_04N)		BE497108
54	At4g17300.1				BF291674

TABLE 3 - *Continued.*

	Arabidopsis	Maize	Rice	*Brachypodium*	Wheat
55	At4g22310.1				BE406897
56	At4g24190.1	AC206514.1-Contig20	AC091774 (Chr_06N)	Bd1(25542766-25542622)	
57	At4g26970.1		AC118132 (Chr_03N)	Bd1(72718547-72718614)	BE443205
58	At4g27880.1	AC203950.2-Contig10	AC105768 (Chr_05N)		
59	At4g28390.1	AC186512.3-Contig12	AP004098 (Chr_02N)	Bd3(54190031-54190267)	
60	At4g28510.1	AC206633.1-Contig38	AP005307 (Chr_07N)	Bd1(1475238-1475040)	
61	At4g33010.1	AC198371.2-Contig31	AP003616 (Chr_06N)	Bd2(48357312-48357500)	
62	At4g34200.1				BF483221
63	At4g35090.1	AC191330.2-Contig32	AC098693 (Chr_03N)	Bd1(25302646-25301866)	BE637476
64	At4g35260.1	AC205538.1-Contig28	OSJN00035 (Chr_04N)	Bd5(17050136-17049892)	
65	At4g35650.1		AP004023 (Chr_02N)		
66	At4g35830.1	AC200293.3-Contig33	AC118132 (Chr_03N)	Bd1(72715257-72715366)	BE443205
67	At4g37040.1	AC210721.1-Contig44			
68	At4g37910.1	AC201993.3-Contig33	AC134237 (Chr_03N)	Bd1(73887106-73886721)	
69	At4g37930.1	AC198482.2-Contig23	AC117988 (Chr_03N)	Bd1(6678592-6678470)	BE425830
70	At4g39660.1	AC203376.2-Contig36	AC126221 (Chr_03N)		
71	At5g03290.1	AC186308.3-Contig36	AP000836 (Chr_01N)	Bd3(44709020-44708713)	BF291357
72	At5g05620.1	AC205034.2-Contig47	AC087425 (Chr_05N)	Bd2(35019318-35018913)	
73	At5g07440.1		OSJN00102 (Chr_04N)	Bd5(20605577-20605793)	
74	At5g08300.1	AC203986.2-Contig28		Bd1(18989307-18989181)	BF200700
75	At5g08530.1	AC199897.2-Contig37	AP003998 (Chr_07N)	Bd1(52156132-52154808)	BE398439
76	At5g08670.1	AC209777.1-Contig67	AP003452 (Chr_01N)	Bd2(47226105-47226262)	BE490596
77	At5g08680.1	AC209777.1-Contig67	AP003452 (Chr_01N)	Bd2(47226770-47227053)	BE490596
78	At5g08690.1	AC209777.1-Contig67	AC129717 (Chr_05N)	Bd2(15404094-15404347)	BE490596
79	At5g09300.1			Bd4(45128041-45127936)	BE403761
80	At5g09590.1	AC201993.3-Contig33	AC134237 (Chr_03N)	Bd1(73887112-73886853)	
81	At5g11770.1	AC210080.1-Contig39	AP003292 (Chr_01N)	Bd2(48702727-48702493)	
82	At5g13420.1	AC210663.1-Contig30	AP004332 (Chr_01N)		
83	At5g13430.1			Bd5(11107474-11107763)	
84	At5g13440.1	AC210663.1-Contig30		Bd5(11107592-11107763)	
85	At5g13490.1	AC190951.3-Contig62	AP004098 (Chr_02N)	Bd3(54188913-54189151)	BE426214
86	At5g14040.1	AC210267.1-Contig67	AP004687 (Chr_06N)	Bd3(57527240-57527551)	
87	At5g14590.1	AC202536.1-Contig33	OSJN00169 (Chr_04N)	Bd5(18576796-18576913)	BE494776
88	At5g26780.1				BE425830
89	At5g37510.1	AC203877.2-Contig146	AC090882 (Chr_03N)	Bd1(8570333-8570048)	
90	At5g39840.1		OSJN00003 (Chr_04N)	Bd5(15838992-15838781)	
91	At5g40810.1	AC211139.1-Contig106	AC093953 (Chr_05N)		BE404540
92	At5g43780.1		CR854994 (Chr_04N)		
93	At5g47040.1	AC212737.1-Contig27			
94	At5g50370.1		AC137922 (Chr_11N)		BE403618
95	At5g50850.1		AC137595 (Chr_09N)	Bd4(40324351-40324475)	
96	At5g50950.1				BE403167
97	At5g53170.1	AC198974.2-Contig46	AP003328 (Chr_01N)	Bd2(44225740-44225612)	
98	At5g56500.1	AC189077.3-Contig113	AP001389 (Chr_06N)		BF146229
99	At5g59370.1	AC202012.3-Contig27	AP003263 (Chr_01N)	Bd4(45810352-45809778)	BE499131
100	At5g59880.1		AC093921 (Chr_05N)		
101	At5g61790.1	AC205801.1-Contig58	OSJN00045 (Chr_04N)	Bd2(920240-919480)	BE490365
102	At5g63400.1			Bd4(44243961-44243863)	BE403618
103	At5g65750.1	AC203429.2-Contig93	OSJN00244 (Chr_04N)	Bd5(10805012-10805263)	
104	At5g66760.1	AC188017.1-Contig8	OSJN01002 (Chr_07N)	Bd1(4910881-4911063)	BQ283248

The second column shows 104 out of the 473 mitochondria-related nuclear genes from *Arabidopsis* presented in different grasses. Columns 3-6 show best matches for maize BAC contigs, rice genome (TIGR version 5), *Brachypodium* Bd21 genome and wheat ESTs. All hits were with Blast score of 100 or higher.

chromosomal rearrangements, such as inversions and translocations. Additionally, functional divergence and neofunctionalization of duplicated genes play an important role in a preferential retention of some duplicated regions. These mechanisms usually lead to some departures from colinearity of orthologous genes across related plant species (PATERSON *et al.*, 2003). Thus considering the age of these polyploidization events and nature of the genomes (maize is completely diploidized with no recognizable genomes while wheat has distinct genomes with chromosomes that can substitute for each other) it is not surprising that the pattern in maize is more complicated than that in wheat.

Clustering analysis of the 11 mapped nuclear genes encoding mitochondria-related proteins identified two clusters on 6.85Mb region of rice. The observed gene clustering and the retention of their syntenic relationship among *Brachypodium*, wheat and in most part maize, emphasizes the importance of these mitochondria-related genes in grasses.

When analyzing the conservation of the larger set of 473 mitochondria-related nuclear genes from *Arabidopsis*, *Brachypodium* seems to be closer to rice (85%) than maize (72%). Additionally, wheat has more mitochondria-related nuclear genes in common with rice (69%) and *Brachypodium* (65%) than with maize (59%). Although wheat sequence data is not as complete as the data available for the sequenced genomes of rice, maize, and *Brachypodium*, relationships among mitochondria-related nuclear genes seem to follow the phylogenetic relationships between these genomes described by others (GPWG, 2001; SALSE and FEUILLET, 2007; BOUCHENAK-KHALLADI *et al.*, 2008).

The low number of the orthologs identified between *Arabidopsis* and other four species (14 to 16%) compared with other studies (ELO *et al.*, 2003; ALEXEYENKO *et al.*, 2006) is likely due to the stringent criterion (Blast similarity score of 100 and higher) applied in our analysis. This criterion had been used by ALEXEYENKO *et al.* (2006) to find duplicated genes in the *Arabidopsis* genome and seemed appropriate for identifying conservation of genes of functional importance. Forty five of the mitochondria-related genes adopted from *Arabidopsis* are conserved across the studied grasses indicating their importance in plant survival. For example, At1g07510.1 is present in all of the grasses and codes for AAA proteases called FtsH10. Number of the AtFtsHs are located in the inner membrane of mitochondria and involved in respiratory function and are essential for oxidative phosphorylation (KOLODZIEJCZAK *et al.,* 2007). Considering polyploidization and extensive diploidization in a number of grass species, specifically maize, conservation of these genes indicates their crucial role in plant development and survival. Additionally, the gene clustering identified during the analysis of rice chromosome 10 could indicate that such clustering might be one of the mechanisms to preserve such important genomic regions from major rearrangements or recombination events that could compromise function of critical genes.

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